Design, synthesis, biophysical and antiprotozoal evaluation of new promising 2,9-*bis*[(substituted-aminomethyl)]-4,7-phenyl-1,10-phenanthroline derivatives by targeting G-quadruplex

Jean Guillon [1,*], Anita Cohen [2], Clotilde Boudot [3], Sarah Monic [1], Solène Savrimoutou [1], Stéphane Moreau [1], Sandra Albenque-Rubio [1], Alexandra Dassonville-Klimpt [4], Jean-Louis Mergny [5], Luisa Ronga [6], Mikel Bernabeu de Maria [6], Eric Largy [7], Valérie Gabelica [7], Serge Moukha [8,9], Pascale Dozolme [8,9], Patrice Agnamey[4], Catherine Mullié [4], Bertrand Courtioux [3] and Pascal Sonnet [4]

[1]Univ. Bordeaux, CNRS, INSERM, ARNA, UMR 5320, U1212, F-33000 Bordeaux, France
[2]Aix-Marseille Univ., IRD, AP-HM, SSA, VITROME, Marseille, France
[3]INSERM U1094, Université de Limoges, Institute of Neuroepidemiology and Tropical Neurology, Limoges, France
[4]Université de Picardie Jules Verne, Agents Infectieux, Résistance et Chimiothérapie (AGIR), UR 4294, UFR de Pharmacie, Amiens, France
[5]Ecole Polytechnique, Laboratoire d'Optique et Biosciences, CNRS, INSERM, Institut Polytechnique de Paris, Palaiseau, France
[6]Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Pau, France
[7]Univ. Bordeaux, CNRS, INSERM, ARNA, UMR 5320, U1212, IECB, F-33600 Pessac, France
[8]Centre de Recherche Cardio-thoracique de Bordeaux (CRCTB), UMR U1045 INSERM, PTIB - Hôpital Xavier Arnozan, F-33600 Pessac, France
[9]INRAE Bordeaux Aquitaine, France

Nowadays, no single tool is available to solve the public health problem of malaria which caused 241 million cases and 627 000 deaths in 2020 worldwide [1]. These indicators are significantly lower than in 2000 (227 million cases and 896 000 deaths) even if there were an estimated 14 million more malaria cases and 47 000 more deaths in 2020 compared to 2019 due to disruptions to services during the pandemic [1]. Nevertheless, the increasing multidrug resistance of *Plasmodium* parasites worldwide is worrying and contributes strongly to WHO's concern that the 2030 targets of the WHO's global malaria strategy will not be met [1]. Among tools, more effective antimalarial medicines with new potential mechanisms of action are urgently required. In this context, efforts to discover new 4-aminoquinoline derivatives are ongoing. Indeed, it is unlikely that the parasite will be able to evolve resistance to drugs targeting the pathway involved in hemoglobin degradation. Previous studies have shown that modification and modulation of the lateral side chain of chloroquine that led to original aminoquinoline compounds avoid the chloroquine resistance mechanism [2]. Another strategy is to design and synthesize quinoline-based drugs that are not recognized by the protein system involved in the drug efflux. Moreover, previous studies on *in vitro* antiplasmodial activity of diaza phenanthrene analogs indicated that the 1,10-phenanthroline skeleton represents an interesting and potential antimalarial lead compound. A few bioactive antimalarial phenanthrolines based on this skeleton have been described such as phenanthrolines C-J (Figure 1).

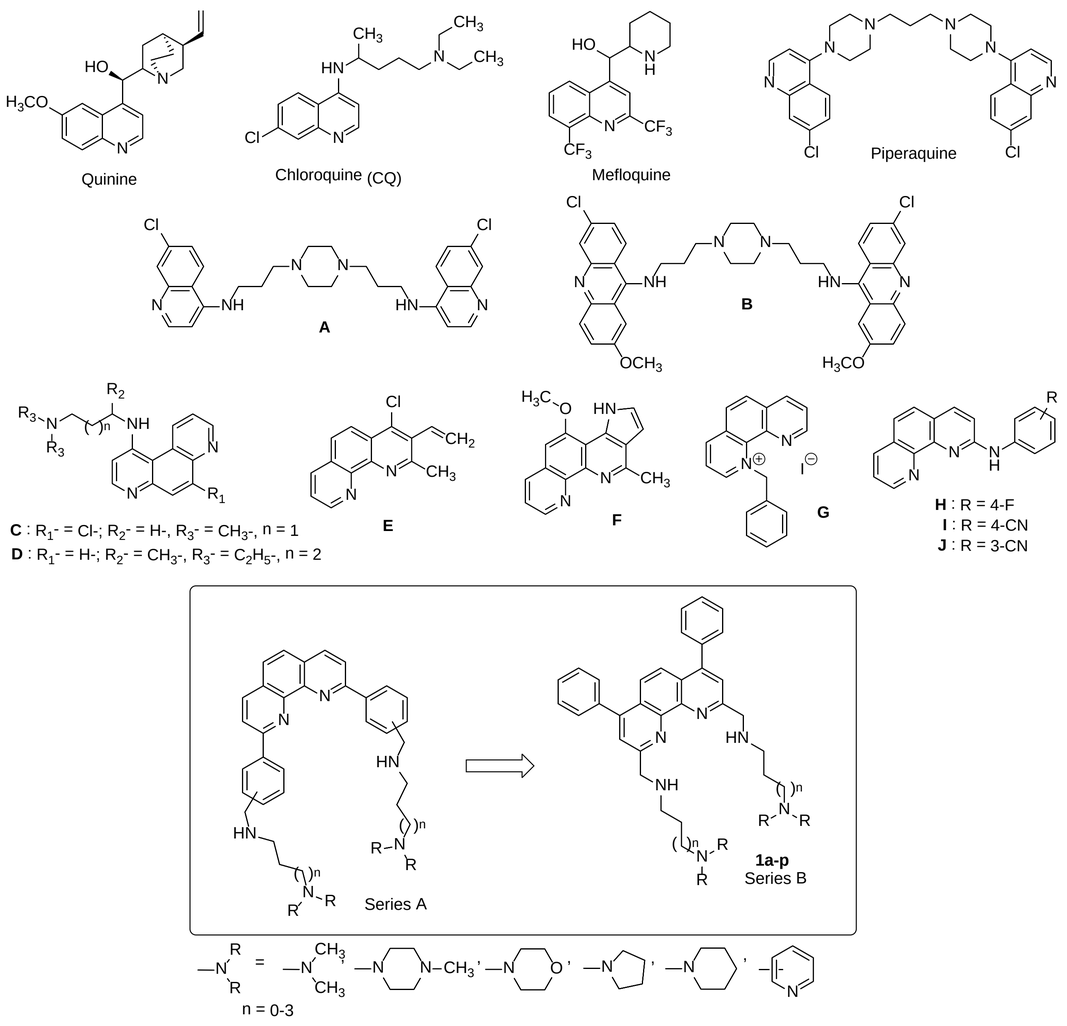
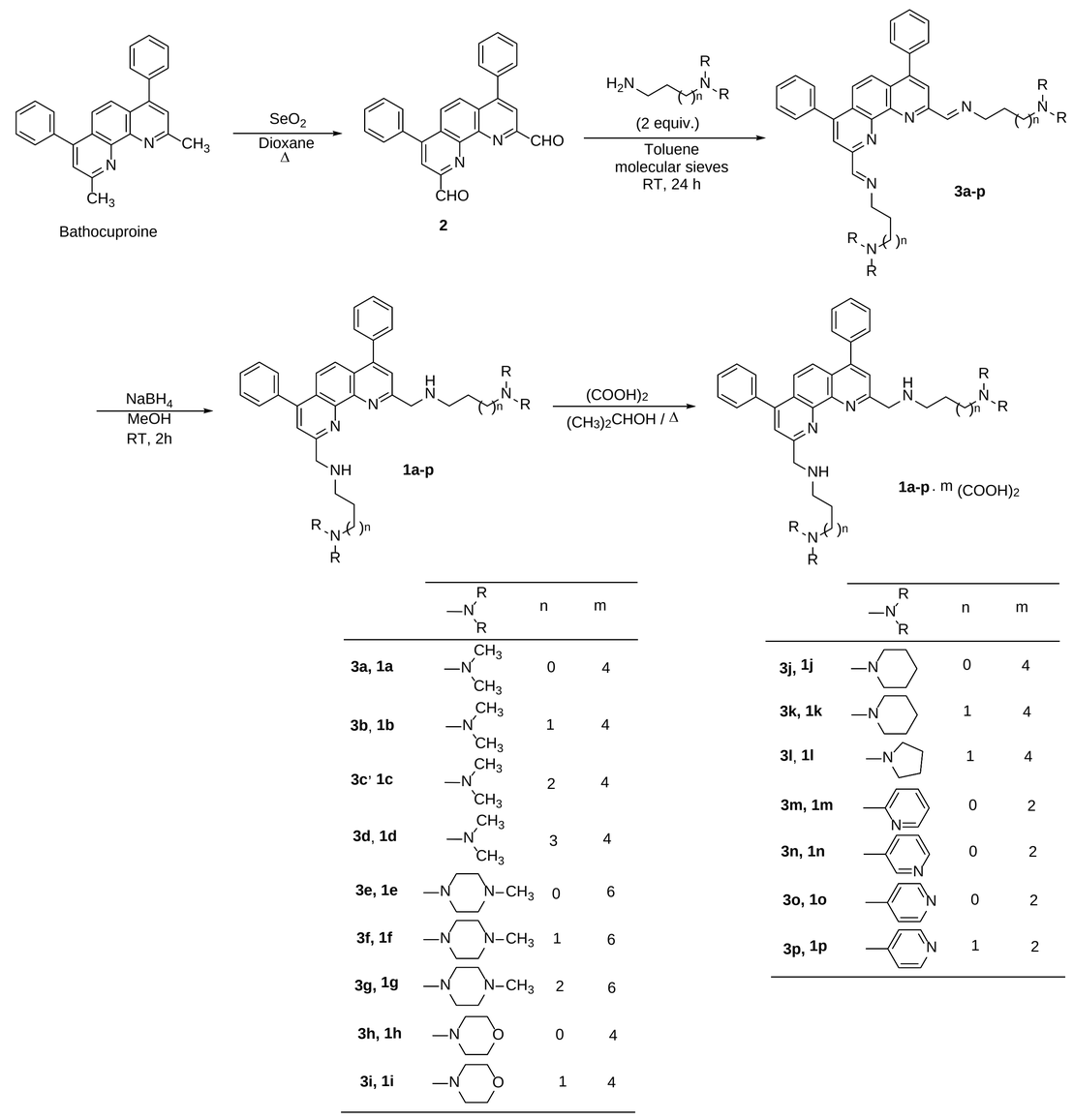


Figure 1. The structures of quinine, chloroquine, mefloquine, piperaquine, bisquinoline A, bisacridine B, 4,7-phenanthrolines C and D, 4-chloro-2-methyl-3-vinyl-1,10-phenanthroline E, 4-methoxy-11-methyl-3*H*-pyrrolo[3,2-*c*][1,10]phenanthroline F, *N*-benzyll-1,10-phenanthroline G, anilinophenanthrolines H-J and newly synthesized substituted phenanthroline derivatives **1**.



		n	m
3a, 1a	$-N(CH_3)_2$	0	4
3b, 1b	$-N(CH_3)_2$	1	4
3c, 1c	$-N(CH_3)_2$	2	4
3d, 1d	$-N(CH_3)_2$	3	4
3e, 1e	$-N\square N-CH_3$	0	6
3f, 1f	$-N\square N-CH_3$	1	6
3g, 1g	$-N\square N-CH_3$	2	6
3h, 1h	$-N\square O$	0	4
3i, 1i	$-N\square O$	1	4

		n	m
3j, 1j	$-N\square$	0	4
3k, 1k	$-N\square$	1	4
3l, 1l	$-N\square$	1	4
3m, 1m	$-\square N$	0	2
3n, 1n	$-\square N$	0	2
3o, 1o	$-\square N$	0	2
3p, 1p	$-\square N$	1	2

Scheme 1. General procedure for the preparation of target compounds **1a-p**.

- During our work focused on the discovery of new nitrogen heterocyclic derivatives that can be used in antiprotozoal chemotherapy, we have previously designed and synthesized a series A of substituted 2,9-bis[(substituted-aminomethyl)phenyl]-1,10-phenanthroline derivatives (Figure 1) designed as antiplasmodial candidates that could bind to *Plasmodium falciparum* DNA G-quadruplexes [2]. Considering our research experience in the field of synthesis of novel heterocyclic antiprotozoan heterocycles, we describe here the conception and the synthesis of new 2,9-bis[(substituted-aminomethyl)]-4,7-phenyl-1,10-phenanthroline derivatives **1** (Series B) (Figure 1) that could be considered as new bio-isoster homologues of our previously synthesized phenanthrolines series A [3]. In this work, we describe their synthesis and their *in vitro* antiplasmodial activity against the chloroquine-resistant (W2) strains and the chloroquine-sensitive (3D7) of the malaria parasite *P. falciparum*. As we could consider that nitrogen heterocyclic pharmacophores are the fundamental moieties of a number of antiprotozoan candidates, these phenanthroline derivatives were also tested for *in vitro* activity against medically important protozoans *Leishmania donovani* and *Trypanosoma brucei brucei*. Indeed, among protozoa of medical interest apart from *Plasmodium*, trypanosomatid parasites are also important. They are responsible for tropical diseases, mainly leishmaniases caused by more than 20 *Leishmania* species described to be infective to humans, and trypanosomiases including human African trypanosomiasis (sleeping sickness) caused by *Trypanosoma brucei* and South American trypanosomiasis (Chagas disease) caused by *Trypanosoma cruzi*. Classified as neglected tropical diseases according to the WHO, which wants to eliminate them as a public health problem by 2030 [4], their impact is even more important because of the current limited therapeutic arsenal which is highly toxic and subject to the development of increasingly drug-resistant parasite strains. Among these new synthesized nitrogen heterocyclic molecules (Scheme 1), a few of them were identified as potential *in vitro* antiplasmodial leads with IC_{50} ranging from 0.03 to 0.73 µM on the W2 and 3D7 strains of *P. falciparum*. Interestingly, the 2,9-bis[3-(pyrrolidin-1-yl)propyl)aminomethyl]-4,7-diphenyl-1,10-phenanthroline **1l** was identified as the most potent and promising antiplasmodial candidate with a ratio of cytotoxic to antiparasitic activities of 505.7 against the *P. falciparum* CQ-resistant strain W2. Against the promastigote forms of *L. donovani*, the phenanthrolines **1h**, **1j, 1n** and **1o** were found the most active compounds with IC_{50} ranging from 2.52 to 4.50 µM. Moreover, the antiprotozoal activity spectrum of our new synthesised phenanthrolines using a *T. brucei brucei* strain revealed IC_{50} values ranging from 0.20 to 1.42 µM, which warrant further investigations. The 2,9-bis[2-(pyridin-4-yl)ethyl)aminomethyl]-4,7-diphenyl-1,10-phenanthroline **1o** was also identified as the most potent trypanosomal candidate with SI of 91 on *Trypanosoma brucei brucei* strain. In addition, the *in vitro* cytotoxicity of these new nitrogen heterocyclic compounds was evaluated on the human HepG2 cell line. Structure-activity relationships of these novel diaza synthetic compounds are here also discussed, as well as their relative ability of targeting *P. falciparum* or *Trypanosoma* telomeres as a hypothetical mechanism of action. In fact, as the telomeres of the parasites could constitute interesting targets, we have also considered the possibility of targeting *Plasmodium* telomeres or *Trypanosoma* chromosomes by stabilizing the *Plasmodium* or *Trypanosoma* G-quadruplexes sequences through FRET melting assays with our bioactive phenanthrolines. Concerning the stabilization of the parasitic G-quadruplex, it could be noticed that the best diphenylphenanthrolines **1** which exhibited an interesting stabilization profile were those substituted by the longest amino-alkylaminomethyl side-chains.

.

[1] World malaria report 2021. Geneva: World Health Organization; **2021**. Licence: CC BY-NC-SA 3.0 IGO. [2] J. Guillon, A. Cohen, R. Nath Das, C. Boudot, N. Meriem Gueddouda, S. Moreau, L. Ronga, S. Savrimoutou, L. Basmaciyan, C. Tisnerat, S. Mestanier, S. Rubio, S. Amaziane, A. Dassonville-Klimpt, N. Azas, B. Courtioux, J.-L. Mergny, C. Mullié, P. Sonnet, Chem. Biol. Drug Des. **2018**, 91, 974. [3] J. Guillon, A. Cohen, C. Boudot, S. Monic, S. Savrimoutou, S. Moreau, S. Albenque-Rubio, A. Dassonville-Klimpt, J.-L. Mergny, L. Ronga, M. Bernabeu de Maria, E. Largy, V. Gabelica, S. Moukha, P. Dozolme, P. Agnamey, C. Mullié, B. Courtioux, P. Sonnet, Pathogens *submitted*. [4] Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva: World Health Organization; **2020**. Licence: CC BY-NC-SA 3.0 IGO.

ECMC 2022
The 8th International Electronic Conference on Medicinal Chemistry
01–30 NOVEMBER 2022 | ONLINE